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T AND B LYMPHOCYTES IN MULTIPLE SCLEROSIS

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SUMMARY

The percentage and total number of E and EAC rosettes, as indicators of T and B lymphocytes respectively, were studied in the blood of subjects with multiple sclerosis (MS) and normals. MS patients in acute exacerbation were found to have a decrease in E rosettes and an increase in EAC rosettes. The relationship of these findings to the pathogenesis of MS is unclear; several possible pathogenetic implications are considered.

INTRODUCTION

The availability of markers for lymphocyte receptors has allowed the investigation of the relative proportion of thymus-dependent (T) and thymus-independent (B) lymphocytes in various neoplastic (Papamichail, Holborow & Keith, 1972; Aisenberg & Block, 1972), immune deficiency (Cooper, Lawton & Bachman, 1971; Cooper, Keightley & Wu, 1974), and putative autoimmune diseases (Papamichail *et al.*, 1972; Williams, DeBoard & Mellbye, 1973; Keith & Curry, 1973; Scheinberg & Cathcart, 1974). In humans, T and B lymphocytes may be distinguished by their capacity to form rosettes with untreated sheep erythrocytes (E) and with sheep erythrocytes sensitized with antibody and complement (EAC), respectively. Multiple sclerosis (MS) is a disease of unknown aetiology in which autoimmunity (Paterson, 1973), broad-based (Davis *et al.*, 1972) or selective immunodeficiency (Ciongoli *et al.*, 1973; Untermohlen & Zabriskie, 1973) and/or viral infection (Weiner, Johnson & Herndon, 1973) have been postulated to play a role in the pathogenesis. We report an analysis of the percentage and absolute numbers of E and EAC rosettes in the peripheral blood of patients with MS.

MATERIALS AND METHODS

Patients. Peripheral blood was obtained from: (a) normal adults ($n = 54$; mean age $30{\cdot}6 \pm 7$ (s.e.m.) years); (b) patients with MS in acute exacerbation ($n = 21$; mean age $29{\cdot}3 \pm 1{\cdot}8$ years); (c) patients with stable MS ($n = 27$; mean age $32{\cdot}7 \pm 1{\cdot}7$ years); (d) patients with amyotrophic lateral sclerosis (ALS) ($n = 4$; mean age $54{\cdot}7 \pm 3{\cdot}1$ years) and; (e) acute occlusive cerebrovascular disease (CVD) ($n = 7$; mean age $66{\cdot}3 \pm 3{\cdot}7$ years).

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Acute multiple sclerosis was defined by the appearance in a definite MS patient of a new symptom or the relatively sudden recurrence of a previous symptom. These patients would fit into the 'relapse' group of McAlpine, Lumsden & Acheson (1965). Blood was obtained during the 3rd to the 14th day of the exacerbation. The stable group was defined as those MS patients with no new symptoms or objective progression of previous symptoms for 6 weeks. These patients would be included in the 'remission' and 'latent' phases of McAlpine *et al.* (1965). None of the subjects had been on corticosteroids for at least 6 weeks prior to the study. Two patients had been treated with azathioprine 2 years before study. None of the patients with cerebrovascular disease had evidence of collagen–vascular disease or were on oral contraceptives, α-methyldopa or hydralazine.

Lymphocyte studies. Differential counts were performed on unseparated whole blood using the usual methods for differential counting. Rosette studies were performed on mononuclear-cell-enriched fractions prepared according to the method of Böyum (1968). E rosettes were assessed using washed sheep red blood cells (SRBC) as previously described by others (Jondal, Holm & Wigzell, 1972). EAC rosettes were studied by methods previously used by us (Abrahamsohn, Nilsson & Abdou, 1974). The rabbit anti-SRBC antibody was a purified IgM globulin fraction (Cordis Laboratories).

RESULTS

Age

There was no difference in the mean age of any of the normals or the MS groups. Both the ALS and CVD groups were significantly older than the normal and MS groups and the CVD groups was older than the ALS group.

E rosettes

The mean percentage of E rosettes was less in the acute exacerbation group ($P<0{\cdot}001$) compared to normals (Table 1). Also, the mean in the acute MS group was less than the stable MS group ($P<0{\cdot}01$) and the stable MS group did not differ significantly from normals. The mean total level of E rosettes was lower in acute MS than in normals, but not significantly so ($P<0{\cdot}1$). Total E rosettes in the stable MS patients were similar to those in normals. The relatively small number of ALS and CVD subjects makes meaningful statistical

TABLE 1. E and EAC lymphocytes in MS and control populations

Subjects	Mean age	E rosettes (%)	Total E rosettes (per mm^3)	EAC rosettes (%)	Total EAC rosettes (per mm^3)
Normals (n = 54)	30·6±0·7	62·6±1·2	1026±44	17·5±0·8	279±20
MS acute exacerbation (n = 21)	29·3±1·8	52·9±2·8	892±84	20·8±1·5	387±51
MS stable (n = 27)	32·2±1·7	60·3±1·2	1059±89	19·2±1·2	273±45
ALS (n = 4)	54·7±3·1	59·5±2·9	1081±101	16·2±2·3	243±62
CVD (n = 7)	66·3±3·7	58·5±2·1	1049±93	19·7±1·3	364±73

comparisons difficult but there is no evidence of a difference from the normal or stable MS group.

EAC rosettes

The percentage of EAC rosettes was significantly greater ($P<0{\cdot}05$) in acute MS when compared with normals. The EAC rosette percentage in the stable MS group was intermediate between the acute MS and the normal group but was not significantly different from either. The mean total number of rosettes was significantly greater in the acute MS group than the mean of the normal ($P<0{\cdot}05$), while the total EAC rosettes in the stable group was intermediate between the two (Table 1). In the instances of ALS and CVD, no definitive statistical conclusion can be made.

DISCUSSION

E and EAC rosettes are widely employed as markers for T and B lymphocytes in man and other species (Shevach, Jaffe & Green, 1973; Ross, Rabellino & Polley 1973; Johansen, Johansen & Talmage, 1974). There appears to be a variable number of mononuclear cells with morphological appearance of lymphocytes which cannot be identified by current cell marker criteria as either T or B lymphocytes (Shevach *et al.*, 1973; Ross *et al.*, 1973; Daniele & Rowlands, 1974). In addition, the use of E and EAC markers alone, preclude relegation of 100% of lymphocytes to T or B cell categories since EAC lymphocytes appear to comprise a subpopulation of total circulating B cells as determined by the presence of immunoglobulin cell surface determinants (Bentwich & Kunkel, 1973; Abrahamsohn *et al.*, 1974).

Our findings indicated a definite decrease in T lymphocytes in the blood of acute MS patients, whereas such a decrease was not found in those with stable disease. At the same time, B cells bearing complement receptors were increased in the blood of acute MS patients. The B cells were increased somewhat but not significantly in the stable MS patients. Recently, others have reported increases in B lymphocytes in MS patients (Arnason, Oger & Kester 1974; Jersild *et al.*, 1975) but the quantitative results of E rosetting studies were not described.

Significant decreases in E rosetting (T) were found in acute exacerbations but not stable MS. Therefore, it seems unlikely that this T-cell aberration is a characteristic of those individuals studied by us who had developed MS. It is possible that the T-cell changes occurred secondary to extensive destruction in the central nervous system (CNS). Antisera reacting with T cells have been experimentally induced by injection of crude brain extracts (Kongshaun *et al.*, 1974) and it is conceivable that an autoimmune response to such elements of CNS tissue could cross-react against T lymphocytes. It has been difficult for us to obtain age-matched subjects with other CNS diseases with patterns of central white matter destruction similar to that seen in MS to investigate this point. In limited studies to date (Table 1) significant decreases in T cells have not been found in such individuals. In MS a T-cell decrease could also conceivably be associated with altered responses to a viral infection (Wybran & Fudenberg, 1973). There is indirect evidence to suggest such altered responses to paramyxoviruses in MS (Ciongoli *et al.*, 1973; Untermohlen & Zabriskie, 1973; Jersild *et al.*, 1975) but *in vivo* and *in vitro* studies (Davis *et al.*, 1972; Jensen, 1968; Frick, Stickl & Zunn, 1974; Lisak *et al.*, 1974) of the status of cell-mediated immunity in MS have yielded conflicting findings. Another possible explanation of this phenomenon is the effect of factor(s)

in the serum of MS patients which either inhibit lymphocyte RNA and/or DNA synthesis (Stjernholm, Wheelock and van den Noorts, 1970; Knowles *et al.*, 1968) or are cytotoxic (Kuwert & Bertrams, 1972) for such cells. One of these factors could selectively inhibit T lymphocyte membrane functions. A fourth possibility is that there is a selective transfer of T lymphocytes from the peripheral blood to inflammatory reaction sites within the CNS during acute exacerbations of disease.

The increases of B cells could conceivably reflect: (1) increased effective lymphocyte activity in MS particularly during acute exacerbations (Bornstein & Appel, 1965; Lisak, Zweiman & Norman, 1975); (2) decreases in supressor T cells allowing for a relative increase in B cells. Other investigators have reported a reciprocal relationship in the relative percentages of T and B cells in some disease states (Dwyer, Bullock & Fields 1973). However, this has not been found by others (Scheinberg & Cathcart, 1974) and indeed, was not the case in some of the subjects reported here. The increase in B cells seen in the small number of patients with CVD might reflect the older mean age of this group (Smith, Evans & Steel, 1974). It is conceivable that the increase in EAC rosetting mononuclear cells found here in acute MS patients might be due, in part, to a contribution by monocytes since the latter cells were not excluded from the incubation mixture (Report of WHO/IARC; Sponsored Workshop on Human B and T Cells, 1974) and have been reported to possess a C3 receptor (Shevach *et al.*, 1973). However, it is of note that the percentage of lymphocytes and monocytes was not different in the blood of any of the groups, and in addition, the mean percentage of 'non-identifiable' mononuclear cells (19–23%) in each of the patient groups studied was the same.

The pathogenic significance of these findings remains undetermined. Additional findings may emerge from sequential studies of individual patients and comparative studies of CSF and blood lymphocytes, where technically feasible.

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